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Technical note on a much simplified method for collecting ruminal fluid using a nylon paint strainer‡§

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Abstract: Collection of ruminal fluid for *in vitro* experimentation has traditionally been accomplished by straining rumen digesta through multiple layers of cheesecloth or other cumbersome filtering materials, such as surgical gauze, nylon tissues or wire gauzes. We here report a comparison of the gross physical, fermentative and microbial characteristics of ruminal fluid collected by straining through cheesecloth or through a nylon mesh paint strainer that, because of its pouched shape, is conveniently filled with digesta thus making the collection and straining process much easier. Whether strained through cheesecloth or the nylon mesh strainer, ruminal fluid did not differ ($P > 0.05$) in dry matter, neutral or acid detergent fiber content or in concentrations of total culturable anaerobes. Total amounts (mM) of volatile fatty acid or ammonia produced after 24 h of incubation of cheesecloth- or nylon mesh-strained ruminal fluid with added tryptose, SigmaCell 50 or starch did not differ ($P > 0.05$) thus indicating that the microbial populations within the strained fluids possessed similar abilities to ferment these test substrates. Because the physical, fermentative and microbial characteristics of the nylon mesh- and cheesecloth-strained ruminal fluid were essentially equivalent, we conclude that the much easier to use nylon mesh strainers can be incorporated into a faster, more convenient ruminal fluid straining method.
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INTRODUCTION

Knowledge of ruminal processes that impact nutrition of the ruminant has been advanced through *in vitro* studies using mixed ruminal microbial populations.[1–3] Such populations, and their activities, have been studied largely through the use of strained ruminal fluid. Moreover, clarified ruminal fluid is often used as a bacteriological media component for certain anaerobic bacteria.[4] The most widely used ruminal fluid collection method has been to strain ruminal contents through multiple layers of cheesecloth or other similar filtering material.[2] Using these materials to strain ruminal contents is cumbersome, often requiring a person to hold the straining material in a funnel or other suitable apparatus while the ruminal digesta is placed onto the material. Once the fluid has been extracted it is impractical to remove the residual digesta from the straining material. In addition, the extended duration required for straining through cheesecloth can allow introduction of considerable amounts of oxygen into the ruminal fluid, which can be detrimental to the growth of strict anaerobes, such as methanogens.[5]

A commercially available nylon mesh paint strainer (Reaves and Co, PO Box 1722, Durham, NC 27 702, USA) has been used in our laboratory as an alternate method of straining ruminal fluid. The nylon mesh paint strainer material has a standard mesh size (124 μm), does not absorb liquid, and has a large opening into a conveniently shaped pouch that obviates many of the disadvantages with cheesecloth by allowing easy and rapid introduction of digesta. The main objective of this study was to compare characteristics of ruminal fluid collected with cheesecloth or the nylon mesh strainer.

MATERIALS AND METHODS

Source of ruminal fluid

Ruminal contents were collected by hand from the ventral sac of three ruminally cannulated Holstein

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cows. The contents were collected from all animals at approximately the same time (between 0800 and 0900 h). Immediately upon removal from the rumen, the contents from each cow were placed into separate pouch-shaped nylon paint strainers or eight-layered cheesecloth and were strained by expressing the fluid into separate insulated containers. When full (approximately 1 liter), the containers were capped and immediately transported to the laboratory where aliquots were apportioned for analysis of physical, fermentative and microbial characteristics. The cows had been fistulated previously and have been maintained in accordance with the Southern Plains Agricultural Research Center Animal Care and Use protocol (ACUC No 00 002). Animals were provided *ad libitum* access to water and minerals and were grazed on a ryegrass pasture during time of ruminal fluid collection.

In vitro incubations

Incubations of ruminal fluid with test substrates were performed by combining the ruminal fluid (1:3) with an anoxic basal medium containing (l^{-1}): 292 mg K_2HPO_4, 202 mg KH_2PO_4, 436 mg NH_4SO_4, 480 mg NaCl, 100 mg $MgSO_4{\cdot}7H_2O$, 64 mg $CaCl_2{\cdot}H_2O$, 4000 mg Na_2CO_3, 600 mg cysteine hydrochloride.[6] The resultant suspensions were transferred to 18 × 150 mm Balch tubes (10 ml per tube) containing either 0.2 g of tryptose, starch or SigmaCell 50. Incubations with undiluted rumen fluid (10 ml) were performed concurrently. Tubes were sealed using rubber stoppers with aluminum crimps and incubated for 24 h at 39 °C under a N_2, CO_2, H_2 (90:5:5 v/v) gas phase. Samples removed after 24 h of incubation were centrifuged (10 000 × *g*, 5 min, 24 °C) and supernatant fluids and cell pellets were separated and frozen for later analysis to determine total VFA and ammonia production and to determine the protein content of the cell pellet. Cell pellets were washed once and resuspended in 9 gl^{-1} NaCl (4×) prior to freezing. For incubations containing added tryptose, samples were also removed after 0, 1 and 3 h of incubation in order to determine the specific activity of ammonia production by the mixed ruminal microbial population. Comparisons of ruminal methane and gas production capabilities were performed following 24 h of incubation of ruminal fluid as above except with 0.2 g of added alfalfa (ground to pass a 1-mm Wiley Mill screen) as substrate and under a CO_2 and H_2 (1:1 v/v) atmosphere. Total gas production was measured through displacement of a glycerol-lubricated syringe.

Analytical

Dry matter, acid detergent fiber and neutral detergent fiber were determined by standard sequential analysis based on the methods of Goering and Van Soest.[7] Ammonia concentrations were determined by the colorimetric method of Chaney and Marbach;[8] cell pellet protein concentrations were determined by the modified Lowry method.[9] Volatile fatty acid concentrations were determined via gas chromatography using a Shimadzu gas chromatograph GC-9A (Shimadzu Corp, Columbia, MD, USA) equipped with a flame ionization detector (Carbopack B-DA, Supelco Inc, Bellefonte, PA, USA) as previously described.[10] Gas composition was analyzed by gas thermal conductivity chromatography (1 ml gas volume) using a Hewlett-Packard (Willmington, DE, USA) model 8690 Chemstation equipped with a flame-ionization detector and using a Carboxen 1000 column (Supelco Inc).

Most probable number (MPN) estimates of total culturable anaerobic bacteria in freshly collected and strained ruminal fluid were determined by a three-tube MPN test.[11] The MPN medium used was anoxic (N_2, CO_2, H_2, 90:5:5 v/v) reinforced clostridial agar supplemented with 1.67 mM xylose, 0.73 mM cellobiose and 460 $ml\,l^{-1}$ filter-sterilized ruminal fluid.[4] Most probable number tubes were incubated at 39 °C for 7 days and growth was determined by measurement of turbidity.[12]

Statistical analysis

Ruminal fluid collected from each cow was considered to be an experimental unit and mean values obtained were analyzed for potential differences between straining materials using a Students' *t* test.[13]

RESULTS AND DISCUSSION

Ruminal fluid samples collected through either cheesecloth or nylon mesh did not differ ($P > 0.05$) in dry matter or in neutral or acid detergent fiber composition (Table 1) indicating similar endogenous substrate composition. Concentrations (MPN) of total culturable anaerobes did not differ ($P > 0.05$) within cheesecloth or nylon mesh strained ruminal fluid (Table 1). Accumulations (mM; mean ± SE) of acetate (60.4 ± 11.5 vs 63.6 ± 1.1), propionate (18.7 ± 1.9 vs 20.2 ± 0.8) or butyrate (8.8 ± 1.7 vs 8.9 ± 0.5) produced during incubation of undiluted cheesecloth or nylon mesh strained fluids, respectively, also did not differ ($P > 0.05$).Thus we conclude that passage of microbes and fermentable substrates through either strainer was equivalent. Total ammonia production from tryptose fermentation was not different ($P > 0.05$); nor was the specific activity of ammonia production from tryptose fermentation (Table 1). Gas production as measured by volume displacement (ml) also did not differ ($P < 0.05$) between filtration methods (Table 1). Total volatile fatty acid production and the ratios of acetate:propionate produced from starch, Sigmacell 50 or tryptose fermentation likewise were not different between cheesecloth- or nylon mesh-strained ruminal fluid ($P > 0.05$) (Table 1).

Collectively, the results presented here indicate that ruminal fluid obtained from filtration by cheesecloth and nylon mesh strainers are indistinguishable for use in *in vitro* fermentations. Nylon mesh strainers are large, pouch-shaped bags that are easily filled with solid ruminal contents. While the amount of methane produced by the fluid obtained by either

Table 1. Microbial, physical and fermentative characteristics of cheesecloth and nylon mesh strained ruminal fluid collected from three cows

	Straining material		
	Cheesecloth	Nylon mesh	*P*
Physical characteristics[a]			
Dry matter ($g\,l^{-1}$)	19.5 ± 4	22.6 ± 0	0.59
Neutral detergent fiber ($g\,kg^{-1}$ DM)	323 ± 27	336 ± 74	0.88
Acid detergent fiber ($g\,kg^{-1}$ DM)	220 ± 5	230 ± 24	0.74
Fermentative characteristics[a]			
NH_3 produced (mM)	77.0 ± 4.2	77.4 ± 11.8	0.98
SAAP ($\mu mol\,mg\ prot^{-1}\ min^{-1}$)[b]	41.0 ± 9.3	41.8 ± 8.8	0.95
Volatile fatty acid produced from typtose (mM)	120.8 ± 1.5	118.5 ± 5.5	0.71
Volatile fatty acid produced from Sigma Cell 50 (mM)	85.9 ± 6.6	81.2 ± 5.5	0.62
Volatile fatty acid produced from starch (mM)	89.7 ± 7.5	71.2 ± 6.2	0.13
Acetate:propionate ratio produced from typtose	3.1 ± 0.1	3.0 ± 0.1	0.57
Acetate:propionate ratio produced from Sigma Cell 50	3.4 ± 0.1	3.2 ± 0.1	0.18
Acetate:propionate ratio produced from starch	3.3 ± 0.1	3.2 ± 0.1	0.53
Total volume gas produced (ml)	17.6 ± 0.4	15.5 ± 0.5	0.09
CH_4 produced ($\mu mol\,ml^{-1}$)	35.9 ± 1.5	34.4 ± 2.3	0.64
Microbial characteristics[a]			
MPN of total culturable anaerobes ($cells\,ml^{-1}$)[c]	$1.4 \pm 0.7 \times 10^{11}$	$1.9 \pm 1.3 \times 10^{11}$	0.76

[a] Values are the mean $\pm$ SEM ($n = 3$).
[b] SAAP, specific activity of ammonia production.
[c] MPN, most probable number.

method did not differ it is reasonable to expect that since filtration is more rapid with the nylon mesh than with cheesecloth (approximately half as much time elapsed), oxygen exposure of strictly anaerobic bacteria and archaea (eg, methanogens) might be reduced using the new method.

Use of a nylon mesh strainer produced ruminal fluid that was indistinguishable in composition and microbial characteristics from that obtained by traditional filtration through cheesecloth. Since these nylon strainers are sturdy, washable and autoclaveable they can be used as a low cost alternative to traditional methods to strain ruminal digesta.

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